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ON THE PROBLEM OF THE FOCAL CHARACTER OF SPRING-AND-SUMMER (TICK) ENCEPHALITIS

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1. INTRODUCTION

Spring-and-summer (tick) encephalitis, first detected in the Far Eastern Province, is an infection which is considerably more widespread than was at first supposed. The Cis-Ural and Volga areas, Vologda, Leningrad and Velikiye Luki Regions /Oblasts/ are likewise districts where during the last five years cases of encephalitis have been detected, and the virus has been extracted both from ixodid ticks and from rodent wild-life (SOLOV'YEV, 1944; CHUMAKOV, MIRONOV, PETROVA, ORLOVA and SHLUGER, 1944).

Naturally the question has come up of the wide distribution of the natural foci of this infection and of the necessity of studying the causes which determine its focal character. The tendency of some of these foci to be concentrated toward areas where ixodid ticks are present in large numbers (POMERANCEV, 1935), the extraction of the virus from ticks in cases where the outbreaks have been studied, and finally the indubitable connection between tick-infestation and the incidence of the disease in humans; all these facts have confirmed the idea of the extremely important role of these arthropods in the propagation of encephalitis among mankind. In some quarters, the close connection has given rise to a belief that the natural-focus character of spring-and-summer encephalitis is completely a function of the presence of infected ixodid ticks. Thus GRASCHENKOV in his address (1943) declared flatly that "the endemic extent of encephalitis is connected with the extent of infection in ticks".

No doubt this statement of the case is correct for understanding the laws of the spreading of encephalitis in humans, but it is hardly adequate for a more profound analysis of the whole problem of the focality of this infection. One must suppose that, as in the case of certain other zoönoses, natural foci of encephalitis may be discovered in areas where the disease is not observed in humans.

This phenomenon of enzootic ity without endemicity is to the highest degree characteristic of a whole list of other infections which are

spread among mankind by ticks and rodents (bubonic plague, tularemia, the leptospiroses, the rickettsioses, tick-borne relapsing fever, cutaneous leishmaniasis); that is, even when there exist channels ensuring the continued circulation of the infection among wild creatures (mammals and arthropods) the channel which would divert it into the human organism may be lacking. The best illustration of this thesis is the discovery of the rickettsia of Rocky Mountain Fever in the ticks infesting hares and rabbits (Haemaphysalis leporis-palustris Say.) in many parts of North America where, thanks to the absence of the characteristic vector Dermacentor andersoni Stiles (Parker, 1935, 1938), the disease has not been observed in humans.

This lends support to the idea that foci of encephalitis may be more widespread in our country than was supposed a few years ago, and we are very definitely confronted with the task of studying the focality of encephalitis. Here it is evident that the solution of the problem must be undertaken by methods somewhat different from those hitherto used. First of all, it is essential that the survey of a focus be regular and unremitting; we must not be satisfied with a short-term expeditionary inspection, as has usually been the case. Indeed it is important to maintain the surveillance of a focus for a number of years, so as to make due allowance for the existence of fluctuations in the numbers of the virus hosts (wild-life and ixodid ticks). No less is it important not to limit ourselves to the survey of any one group of ectoparasites playing an important part in the transmission of the infection to man (the ixodid ticks), but to pay attention to other arthropods capable of carrying the infection from one wild creature to another, thus promoting the circulation of the virus within the focus.

> Thus it is particularly important to investigate the gamasid mites, which obviously play a considerable part in transmitting both tularemia and the rickettsial diseases from rodent to rodent (SIMMONS, 1941).
>
> Likewise chiggers (Trombiculidae), which transmit acute infections like tsutsugamushi fever to humans, may also play a part in spreading virus infections among the wild-life of a focus. Finally we might also follow up the matter of whether one link of the chain of propagation might not be the flea, which plays such an important role in the epidemiology and epizootology of bubonic plague, tularemia and the rickettsial diseases (IOFF, 1944; SIMMONS, 1941; OLSUF'YEV, 1940).

In carrying out a detailed survey of this kind at any one focus, we should also at the same time investigate [the whole of] whatever area is known to have an encephalitis tendency, both in order to establish whether [the whole of] it may not constitute a potential focus of infection, and also to chart the movement of the rodent population and its ectoparasites, within the focus and outside it.

2. PLACE AND METHOD OF SURVEY

A focus was investigated in the Nelidovo District [Nelidovski Raion] of Velikiye Luki Region [Oblast']. Here, in the triangle formed by

Faniklia Siding and the villages of Niva and Gora, cases of encephalitis were observed in the summer of 1942 among military units stationed in the forest. It was at this spot that I.M.OLIGER in the summer of 1943 collected ixodid ticks, from which encephalitis virus was extracted in M.P.CHUMAKOV's laboratory. No cases of encephalitis had been observed among the civilian population before the war, nor were any observed in 1942, 1943 and 1944.*

As a control area we selected the vicinity of Avdotino Village of Semenov Rural Soviet, Mikhnev District, Moscow Region. Our choice of this place was based on the fact that the existence of a hospital here for the past 40-odd years, and of a Tularemia Station since 1938, would have shown up any cases of encephalitis during recent years if any had occurred.

The collecting and counting of small mammals and their ectoparasites in the focus-area was carried out by N.I.KALABUKHOV; in the Mikhnev District by him and Assistant A.N.NAIDENOVA.

For capturing the animals we used the "Hero" mouse-trap and other non-killing traps. The count in each survey was handled by setting usually 40 to 50 traps and keeping the reckoning for a four to five day period, so as to have not less than 200 trap-hours. The traps were patrolled (and bait changed) twice a day, A.M. and P.M. The animals caught were put in bags to ensure all ectoparasites being collected, then, at the Niva Village travelling laboratory and at the Tularemia Station in the town of Semenovskoye, the animals were looked over and a count made of ectoparasites found, which we either collected alive (ixodid ticks, gamasids and fleas) or preserved in 50% glycerine (chiggers.)

Here too we dissected the animals, excising the brain and preserving it in 80% glycerine. From then on the brains were kept in insulated containers on ice. Upon arrival of the specimens at Moscow, the parasites were identified. The species-classification of the ticks was undertaken by A.B.LANGE, a graduate worker in the Faculty of Entomology of Moscow State University, under the direction of Professor A.A.ZAKHVATKIN. The fleas were identified by Doctor I.G.IOFF and V.A. TIFLOV (Stavropol' Anti-bubonic Station).

Then the collected specimens were examined for virus content according to the usual technique (SOLOV'YEV, 1944) under the direction of A.K.SHUBLADZE at the Virus Division of the Headquarters of the Institute of Experimental Medicine.

Between December 1943 and January 1945, eleven surveys were carried out, six of them in the focus-area and five in the control area.

* One case, clinically diagnosed as encephalitis at the Nelidovo hospital at the beginning of June 1944, was not given a virusological or epidemiological examination, since we were not on the spot at the time.

A) In the focus-area: 19th to 30th January; 20 to 29th March; 20th to 30th May; 18th to 27th July; 21st to 29th September; 23rd to 31st January, 1945.

B) Outside the focus: 11th to 18th December, 1943; 21st to 26th February; 6th to 12th July; 6th to 12th September; 17th to 22nd November, 1944.

Thus the survey covered the whole annual cycle of the life of these mammals and their ectoparasites. However, we were unable, for a number of reasons, to carry out the survey in the control area at the beginning of spring, the period when the ixodid ticks come out of hibernation, and at the focus we could not carry out the survey at the beginning of winter. Curves of the species-composition and population-figures for the mammals and the ectoparasites, in the focus-area and in the control area, are given below.

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3. SPECIES-COMPOSITION AND POPULATION, SMALL MAMMALS

Our findings on the species-composition of the small mammalian fauna, in the forest at the focus and in the control area, are presented in tables 1, 2, and 3 and in figure 1.

Figure 1 gives the census for the forest near Avdotino Village, shown in percent of the number of trap-hours. In Table 2 we give figures for the catch in the focus-area; that is, in a portion of the forest in the shape of a triangle formed by the villages of Gora and Niva and the road running to Paniklia Siding on the railroad.

Data on the relative numbers of the basic types of animals are given in figure 1 (as percentages of the number of trap-hours).

Assuming that the yellow-necked mouse Apodemus flavicollis Melch. must exist in the forest, a species which is a virus-vector in the Volga Country (SOLOV'YEV, 1944), and knowing that in winter this species of rodent usually moves into settlements, we simultaneously undertook to trap the animals in the houses of Niva Village too.

The figures for this census, which are of indubitable interest, are given in table 3.

From figure 1 it will be seen that the cycles of rodent population in the two areas coincide.

Obviously, the steady growth of the rodent population towards autumn is of real epizootological significance; it was from specimens obtained in September that we extracted two strains of the virus (vide infra).

Table 1. Census of small mammals in the forest near Avdotino Village, Semenov Rural Soviet, Mikhnev District, Moscow Region, 1943-44.

Survey data	14th to 18th Jan 1943	1944				Totals
		21st to 26th Feb.	6th to 12th July	6th to 12th Sept.	17th to 22nd Nov.	
Number of trap-hours	216	208	284	237	213	1158
Number of animals caught	24	21	42	94	24	205
Breakdown:						
Shrew (Sorex araneus)	3	-	1	4	1	9
Red vole (Evotomys glareolus)	11	13	25	59	14	122
Dark vole (Microtus agrestis)	1	1	6	2	-	10
Common vole (Microtus arvalis*)	-	1	3	2	-	6
Wood-mouse (Apodemus sylvaticus)	9	6	7	26	8	56
Yellow-neck mouse (Apodemus flavicollis)	-	-	-	-	1	1

* In addition, ectoparasite and brains were collected from 161 common voles (Microtus arvalis) caught in hay-ricks and on the fields near Avdotino Village [Dec., 1943, 85 specimens; Feb., 1944, 64 specimens; Nov., 1944, 12 specimens].

Table 2. Small rodent population in forest in focal area. [Zhigliçki Rural Soviet, Nelidovo District, Velikiye Luki Region]

Survey data	1944 Jan. 19-30	Mar. 20-29	May 3-30	July 18-27	Sept. 21-29	Jan. 23-31 1945	Totals
Number of trap-hours	324	282	430	263	203	228	1730
Number of animals caught	17	24	21	43	75	13	193
Breakdown:							
Common shrew (Sorex araneus L.)	1	4	-	14	5	5	29
Middle-sized shrew (S.macropygmaeus Pall.)	-	-	-	1	-	-	1
Red vole (Evotomys glareolus Schr.)	10	16	19	24	61	8	138
Dark vole (Microtus agrestis Pall).	3	4	-	4	1	-	12
Common vole (Microtus arvalis Pall.)	3	-	-	-	2	-	5 *
Field-mouse (Apodemus agrarius Pall.)	-	-	1	-	1	-	2
Yellow-neck mouse (Apodemus flavicollis Melch.)	-	-	1	-	5	-	6

* Also investigated were 15 specimens of Microtus arvalis Pall. caught in the fields near Niva Village (July 4th, 1944 and Sept. 11, 1944) and one hare (Lepus europaeus Pall.) shot at the same place on Jan. 22nd, 1944.

Table 3. Catch of rodents in buildings of Niva Village (Nelidovo District, Velikiye Luki Region)

Period of Census	Jan.-Mar 1944 and Jan,1945	May-July-Sept,1944	Totals
Number of trap-hours	120	83	203
Total of animals caught	16	13	29
Breakdown:			
Domestic mouse (Mus musculus L.)	5	9	14
Yellow-neck mouse (A.flavicollis Melch.)	6	-	6
Grey rat (Rattus norvegicus Berkenh.)	3	-	3
Black rat (Rattus rattus rattus L. and R. r. alexandrinus Geoffr.)	2	4	6

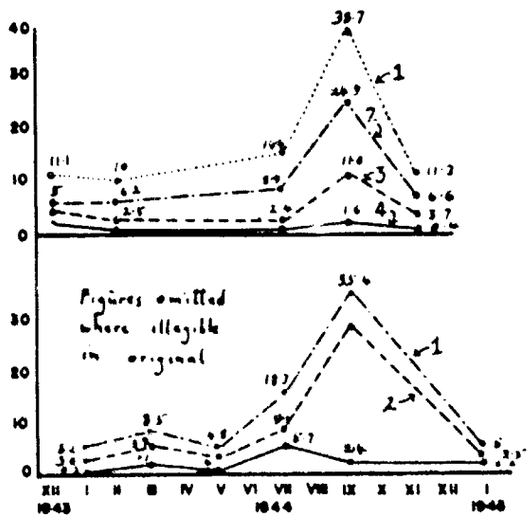

Fig. 2. Seasonal population-curves of basic species of small mammals.

Legend: 1 - All species. 2 - Evotomys. 3 - Apodemus sylvaticus. 4 - Sorex.

Above: encephalitis-free district.
Below: focus-area.

4. SPECIES-COMPOSITION AND POPULATION, ECTOPARASITES

The results of our survey of mice and sh. .w at different seasons of the year and our findings re the extent of their infestation with ectoparasites are given in Table 4 and Figure 2. If we now turn to the details of the species-composition of the parasites of the small mammals in the areas surveyed, we find first of all the ixodid tick. Ixodidae collected from rodents are almost exclusively in the larval or nymph stage (POMERANÇEV, 1935; OLSUF'YEV, 1940). This makes it in practice almost impossible to determine their species. However, from isolated specimens of males and females found on rodents, cattle and plants, it was possible to establish that four species of tick are found in the focus-area.

1) Ixodes ricinus L.
2) Ixodes persulcatus Sch. } Found on vegetation, on cattle and on humans.

3) Ixodes trianguliceps Birul. { One female on Evotomys glareolus, May 29, 1944.

4) Dermacentor sp. (imago not found).

In Mikhnev District, where the ixodid tick population was generally speaking extremely small, we found:-

1) Ixodes ricinus L.

2) Dermacentor pictus Herm.

For the solution of the problem with which we are concerned, a very interesting point is the discovery of three specimens of Ixodes coexisting in the focus-area: the widespread Ixodes ricinus L., then Ixodes persulcatus Sch., known to be a carrier of encephalitis (SOLOV'YEV, 1944; CHUMAKOV, MIRONOV, ORLOVA, PETROVA and SHLUGER, 1944), and finally Ixodes trianguliceps Birul., infesting rodents and likewise carrying the encephalitis virus (SOLOV'YEV, 1944). The last-named species was discovered in earlier collections from Mikhnev District too, made by N.G.OLSUF'YEV (1940), although we did not find it in our surveys.

There was a very regular infestation of the rodents with chiggers, the Trombiculidae. * Particularly numerous (as many as 50 to 100 individuals per head) on the red voles (Figure 2), these chiggers were found only as isolated individuals on Microtus agrestis and only occasionally on other species of forest mammals. We also note that the degree of tick-infestation of mammals of all species was higher in the focus-area than in the "free area (Figure 2).

The degree of infestation of the animals with fleas was for us an exceedingly important piece of information, because it was from these ectoparasites of the focus that one of the strains of the virus was extracted, in September 1944. The virus was discovered in fleas collected from red

* Both inside the focus-area and outside, the same species of Trombiculid was found on the rodents (as determined by Ye.S.SHLUGER).

Table 4. Ectoparasites collected from small mammals

Ectoparasites \ Date of collection	At focus		In control area	
	Jan-March 1944 Jan.,1944	May-Sept 1944	Dec,1943 Feb-Sept 1944	July-Sept 1944
Ticks (Ixodidae)	1 (larva)	132 including 5 imagos	2 (larvae)	4
Gamasoidea	155	170	173	152
Chiggers (Trombiculidae)	579	1974	435	460
Fleas (Aphaniptera)	70	82	72	62

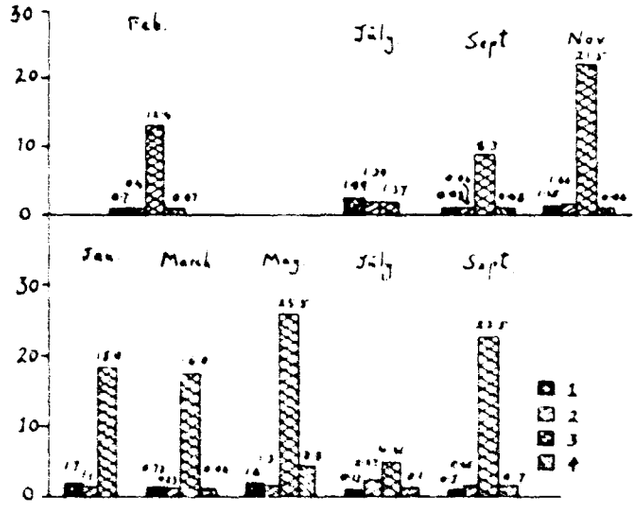

Fig. 2. Degree of infestation with ticks, voles Evotomys glareolus Schr.

Legend: Vertically, mean number of ectoparasites per vole; 1 - fleas, 2 - gamasids, 3 - chiggers, 4 - ixodidae

Above - encephalitis-free area.
Below - focus-area.

voles (count on 16 individuals). We should mention that on the rodents in the focus-area the following eight species of fleas were found:

1) Ctenophthalmus agyrtes. } These are the principal species
2) Ctenophthalmus uncinatus. }
3) Ceratophyllus penicilliger. } Isolated specimens of these
4) Ceratophyllus turbidus.
5) Ctenopsyllus segnis.
6) Ctenopsyllus bidentatus.
7) Rhadinopsylla intellega.
8) Hystrichopsylla talpae

In the control area, we found the three first-mentioned and the two last-mentioned species of flea, also isolated specimens of Ceratophyllus sciurorum, Amphipsylla rossica, Ctenopsyllus sylvaticus, Ctenophthalmus assimilis and Doratopsylla dasycnemus.

5. RESULTS OF VIRUSOLOGICAL TESTS

427 brains of different rodents were tested, in batches of three or four, and 4028 specimens of the ectoparasites, in batches of 4 to 20. The test-material from each batch was passaged intracerebrally through white mice not less than three times. In one case, a strain of virus was extracted on the third passage from the brain of wood-mice (Apodemus sylvaticus) caught outside the encephalitis focus. The other strain of the virus was extracted, likewise on the third passage, from fleas collected from red voles (Evotomys glareolus) caught inside the focus. Both the strains thus obtained were passaged ten times through white mice, with 100% positive results. This intracerebral infection induced in the mice symptoms of injury to the nervous system of varying degrees of severity, in the form of tremors and convulsions. In the animals infected with the virus extracted from the wood-mice, various paralyses of the hind limbs were the predominant symptom. On the other hand the virus extracted from fleas did not as a rule cause paralysis in mice on the first passages. Assuming different type-specific immune sera, we made serological identification tests with other neuroviruses to identify the strains we had obtained. For tests on the neutralization of the wood-mouse and flea viruses, we used the following sera: horse and rabbit sera hyperimmune to the virus of spring-and-summer encephalitis; serum from a person suffering from a chronic form of diffuse sclerosis; dog serum hyperimmune to the virus of silver fox encephalitis; rabbit serum hyperimmune to the virus of equine encephalomyelitis, and sheep serum immune to the virus of "louping ill". In all cases the virus extracted from the wood-mice was neutralized only with sera specific for the virus of spring-and-summer encephalitis.

Patient convalescing after spring-summer encephalitis	+	-
Patient with chronic diffuse sclerosis	-	-
Horse hyperimmune to spring-summer encephalitis	+	-
Rabbit hyperimmune to spring-summer	+	-
Rabbit hyperimmune to equine encephalomyelitis	-	±
Dog hyperimmune to silver fox encephalitis	-	-
Sheep immune to louping ill.	-	±
Rabbit with normal serum	-	-

The virus extracted from fleas was only feebly neutralized by serum from rabbits immunized with the virus of equine encephalomyelitis and by serum from sheep immunized to louping ill. Legend /in Table 57:-

\+ Complete neutralization of the virus.

± Weak neutralization of the virus.

\- No neutralization.

Summing up our experiments on the serological identification of the viruses under study, it is in order to suggest that the virus extracted from wood-mice is a strain of spring-and-summer encephalitis virus; the nature of the second virus, extracted from fleas, remains uncertain that study thereof will be continued in further, more broadly based researches.

CONCLUSIONS

The data we have obtained are of real significance for the study of the problem of the focality of tick encephalitis. The first of the facts which are of inescapable interest to us is the detection of the tick encephalitis virus in the brains of wood-mice from the control area, where

cases of encephalitis had not been observed. This fact once more demonstrates that the reason for the "freedom" of such an area is the absence of contact of the local population with the source of the infection (perhaps because of the absence here of that tick-species which is prone to attack man, namely Ixodes persulcatus) or the existence of a natural immunity in the local population (SOLOV'YEV, 1944).

The extraction of the virus from wood-mice, animals which SOLOV'YEV (1944) did not include in his list of spontaneous virus-vectors, is nevertheless not so unexpected, since in the reports of the work of the Academy of Sciences' Kazakhstan Branch there was recently published a paper by GALUZO (1943) in which he mentions that he was successful in extracting the virus of Alma-Ata encephalitis from the brain of this species. It seems to us that these findings do not really contradict SOLOV'YEV's conclusion (1944) that wood-mice are not susceptible to encephalitis virus; on the contrary these findings impress upon us that a low susceptibility of some species to this infection in no way prevents the maintenance of the infection in the focus.

Of great interest is the discovery of a virus (to be sure, one which has not yet been identified with the virus of tick encephalitis) in fleas from the red vole Evotomys glareolus Schr. caught in the focal area. This finding, this enlargement of the circle of possible vectors of the virus, is not only of interest for the solution of a number of problems in the epidemiology of this infection; it also points out the possible propagation-route of the disease in wild creatures which are not infested with ixodid ticks, for instance the propagation of silver fox encephalitis in animal-breeding nurseries or of marmot encephalitis in the high mountain regions of Kazakhstan (GALUZO, 1943). The discovery of Ixodes trianguliceps Birula on rodents inside and outside the focus also compels us to agree with the thesis that the propagation of encephalitis among the smaller animals is perhaps closely connected with the presence of this species of tick, as suggested by SOLOV'YEV (1944).

The survey sets us a large number of other interesting problems, the answers to which it will be possible to give only after the researches here begun have been continued on a more thorough-going and persistent basis.

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* Name here given as ORLOV, but it appears twice in text as ORLOVA. (Tr.)